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SOME FACTORS CONTROLLING REPRODUCTION IN THE SPIDER CRAB, *LIBINIA EMARGINATA* [1]

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While oogenesis in crustaceans has frequently been studied, detailed knowledge of the anatomy, histology and hormonal controls involved in reproduction in female brachyurans have been limited (see Ryan, 1967; Adiyodi and Adiyodi, 1970 for review). The structure and function of the reproductive system in the crab *Portunus* during the molting and reproductive cycles of the preadult and two adult instars has been examined recently (Ryan, 1967). Although a definite relationship exists between the molting and reproductive cycles in crustacean females, little is known regarding this relationship between molting and sexual maturation among the members of the family Majidae to which *Libinia* belongs. For instance, *Maja* is reported to undergo a final or terminal molt (Drach, 1939). Whether this occurs in all Oxyrhyncha is not known.

This study was undertaken to investigate the relationship between molting and sexual maturation and to determine what if any hormonal controls may be operating in ovarian development in the female spider crab, *Libinia emarginata*.

Materials and Methods

Immature and mature female specimens of *Libinia* were collected by the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachusetts during the summers of 1967–1970. The crabs were maintained in aquaria with running sea water and fed periodically on *Mytilus* or *Spisula*. Carapace length of all crabs studied was measured with calipers.

The eyestalks of immature and mature female crabs were removed at their base with a dissecting needle and scissors to induce molting. No special measures were needed to control or prevent excessive bleeding. In addition, mature females with or without broods were subjected to eyestalk ablation and the effects on reproduction were studied. Control animals (non de-eyestalked) were kept in adjacent aquaria. Males were placed in all aquaria.

Reproductive tracts of both immature and mature females were dissected for study. The ovaries were fixed in calcium-formol, dehydrated and embedded in paraffin for light microscopy or were fixed in Karnovsky's (1965) paraformaldehyde-glutaraldehyde fixative. The latter were postfixed in 1% OsO_4, dehydrated

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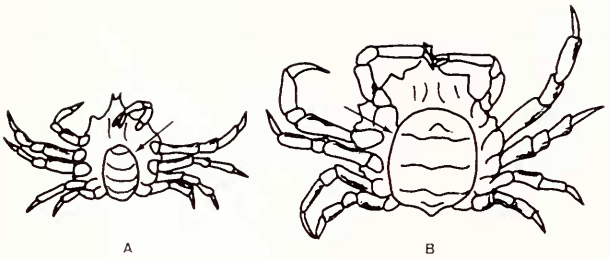

FIGURE 1. Ventral view of immature (A) and mature (B) female *Libinia*. Note difference in outline of abdomen (arrows), × 1/2.

and embedded in Araldite for electron microscopy. Paraffin sections (5 μ) or thick plastic sections (1 μ) were stained with toluidine blue to determine the extent of ovarian differentiation at various stages.

OBSERVATIONS

Sexual maturation and molting

Classification of females as immature or mature was determined on the basis of size and shape of their abdomens. The abdomen of immature females is narrow and does not extend to the base of the legs (Fig. 1). In the adult, it is rounded and almost reaches to the base of the legs (Fig. 1). Figure 2 indicates the carapace length in centimeters of immature and mature females. The maximum length of immature females seen from the wild populations was 6.0 cm. Mature females

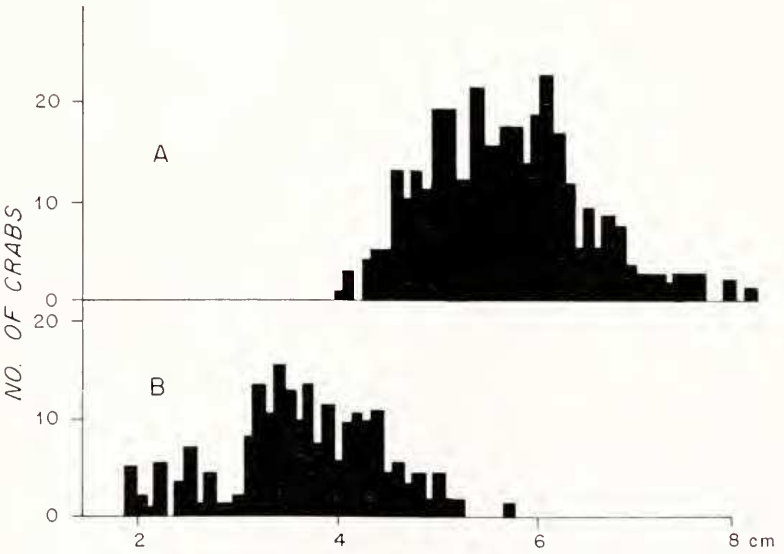

FIGURE 2. Carapace length of individual crabs. A indicates the length of 318 mature females, B—the length of 202 immature females.

exceed 4.0 cm. Thus, in the 4–6 cm range, females may be either mature or immature.

Among the immature spider crabs studied (above 1.9 cm carapace length), molting normally was observed to begin in August or September. The time varied from middle August during the summer of 1968 to the middle of September in 1970. Successful molting occurred even when large numbers of crabs occupied the same aquarium. Carapace length of several immature control females prior to molt and length and sexual state after a normal molt are shown in Figure 3. Crabs undergoing normal ecdysis vary in the amount of growth following the molt as well as the state of sexual maturation. No mature females were ever observed undergoing ecdysis.

Immature and mature females were destalked and observed for indications of molting. None of the 43 mature destalked females molted or upon dissection showed signs of molting. Among the 51 immature females which were destalked in late July or August, most began molting within two weeks after the operation. Those destalked in May or June showed no signs of molting within two weeks. Molt, however, was initiated after four to five weeks. Destalked crabs had difficulty in completing the molting process and although the number of crabs per aquarium was reduced (10 per aquarium), a molting crab frequently was attacked

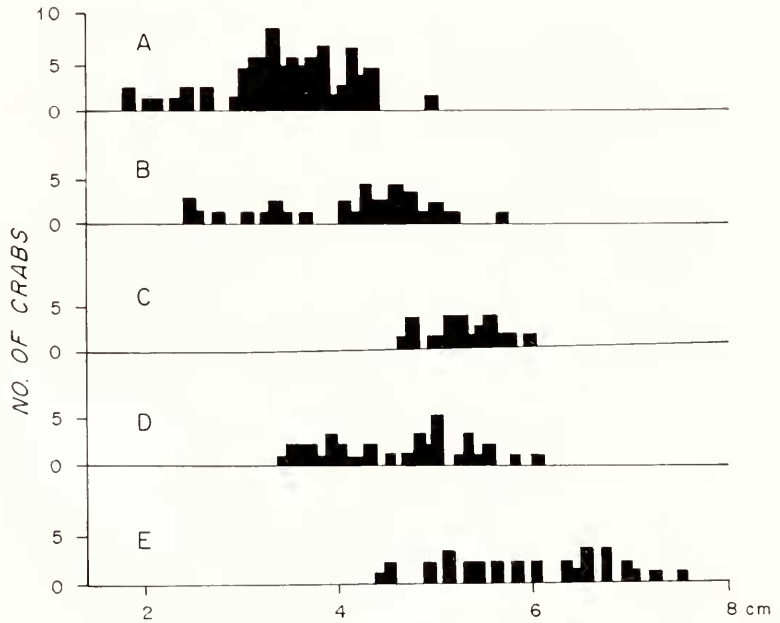

FIGURE 3. A–C, carapace length of intact females; A—carapace length of 76 immature control female crabs, B—Thirty seven females which remained immature following a normal molt, C—Twenty one females which molted to maturity, D–E Destalked immature females. In D, the carapace length (in centimeters) prior to the molt of 38 immature females is indicated. In E, the carapace length of 32 recovered females all of which retained the immature apron.

and eaten by her aquarium mates. Such behavior was not seen in molting, normal, unoperated crabs even under crowded conditions (30–40 per aquarium). In addition, destalked crabs frequently decorated their carapaces with bits of shell, sea urchin spines, and other debris found on the aquarium bottom. Unoperated crabs rarely did such in these experiments.

As reported earlier (Hinsch, 1970), destalked immature females rarely molted to the mature state. This was particularly true when immature females of various sizes were destalked several weeks prior to the time when their controls commenced to molt naturally. Giant immature females were produced. As shown in Figure 3, one might have expected on the basis of carapace length alone (compare with Fig. 2) that several of these crabs would have been mature. However, immature females which were destalked and which molted at the same time as their controls frequently molted to maturity. Two destalked females who had molted once following the operation underwent an additional molt two weeks after the first. This molt was fatal. The abdomen and ovaries of these two crabs were those of an immature female.

TABLE I

Destalking experiments of mature female Libinia. The experimental females were destalked on August 7, 1969 and observed periodically thereafter. Oviposition was stimulated in the operated females

	Egg mass/No. of crabs						
	Aug. 11	Aug. 18	Aug. 26	Sept. 2	Sept. 11	Sept. 15	Sept. 23
Experimentals (destalked)							
20 with eggs in brood pouch	19/19	10/19	14/16	14/15	6/15	9/15	10/15
16 without eggs in brood pouch	0/14	0/14	11/12	9/12	5/12	8/11	9/9
Controls							
15 (eyestalks not removed)	2/15	3/14	3/13	1/14	0/14	0/14	0/14

Reproductive cycles

The breeding behavior and reproductive cycles of the female *Libinia* have been described (Hinsch, 1968). Mature females apparently oviposit for the first time in late May or early June. This brood is often quite small. It is followed by additional spawnings at 25 days intervals. The time of last oviposition varies seasonally.

To determine the role of the eyestalk hormones on reproduction, mature females were destalked during various stages of their ovarian cycles. Following the operation, the crabs were placed in aquaria with some males and observed. The 25 day brood period was unaltered and the females continued to attract males at the time of zoeae hatching as did those in the control tanks (Hinsch, 1968).

In July 1968, 43 mature females with broods were destalked. On September 23, 24 of the 27 survivors had orange egg masses (new). Among the wild population, only 3 of 93 mature females carried broods at this time.

In early August 1969, mature females with broods and females who had been without broods for at least one week were subjected to destalking and observed periodically (Table I). By late September, only females which had been destalked carried egg masses in their brood pouches.

On September 3, 1970, these experiments were repeated on an additional 130 crabs. Seventy females with broods, many of them relatively new (orange egg masses), were used as controls. Sixty females lacking broods were separated into two groups. Thirty were left unoperated and placed in an aquarium with males. The other thirty were destalked prior to being placed with males in another aquarium. All crabs were observed periodically and the presence or absence of a brood noted (Table II). The operated females were frequently observed mating with males, while unoperated females rarely mated at this time of the year. On September 26, 27 of the 28 surviving operated females contained orange (new) egg masses in their brood pouches. Of the 99 unoperated females, 5 had new (orange) egg masses, 5 had brown egg masses containing zoeae about to hatch and 88 were without egg masses. As noted earlier (Hinsch, 1968) a female need not mate immediately before each brood of eggs is laid to produce viable young.

TABLE II

Presence or absence of egg masses in the brood pouches was observed in mature females. As indicated, oviposition was stimulated in the females which have been destalked

(1970)	Egg masses/No. of crabs		
	Sept. 3	Sept. 21	Sept. 26
Experimental females (destalked)	0/30	14/29	27/28
Control females (non-destalked)			
Females with broods	70/70	12/70	10/70
Females without broods	0/30	1/29	1/29

Ovarian development

Dissection of immature females revealed ovaries which were small, white H-shaped organs. Sections of these ovaries showed large masses of immature oocytes near a central core of syncytial cells and showed no signs of vitellogenesis (Fig. 4). The ovaries of females which have just molted to maturity are also white and only slightly larger than those of immature females. These oocytes are small, have vacuolated nucleoli and no yolk. One month following the molt to maturity, many of the crabs have ovaries which are enlarged, orange in color and have oocytes in which vitellogenesis is well advanced. Following oviposition, ovarian development takes place in the ovary of the mature female as she is brooding her young. Eggs collected near the end of a brooding cycle have well developed yolk and a forming egg coat (Fig. 5). Females who are about to release zoeae during the breeding season have oocytes in their ovaries which are fully developed, surrounded by an egg envelope and lack follicle cells. As each brood hatches, a new mass of eggs is generally oviposited after approximately 6 to 12 hours (Hinsch, 1968). Ovaries of females at the end of the breeding season vary in stage of maturation, although

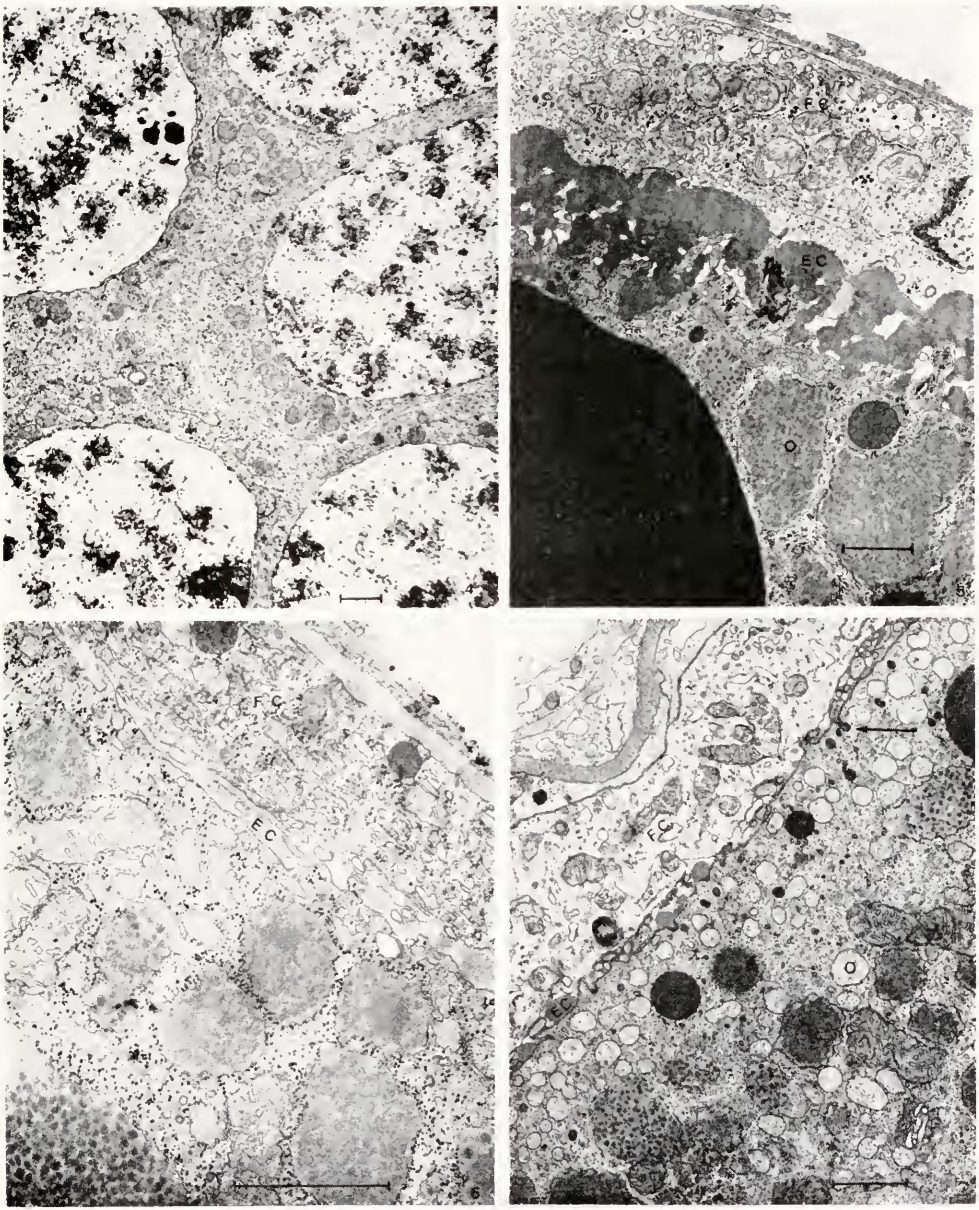

FIGURE 4. Ovary from an immature female collected in mid-August. Premolt condition is unknown. Many small oocytes are apparent.

FIGURE 5. Oocyte from mature female brooding young. The oocyte (O) is surrounded by forming egg coat (EC) and follicle cells (FC).

FIGURE 6. Mature oocytes from bright orange ovary of female at the end of the breeding season. Follicle cells (FC) still surround the oocyte and egg coat (EC) has only partially formed.

FIGURE 7. Oocyte from orange ovary of a mature female in mid-November. In this instance vitellogenesis including pinocytosis (arrow) is seen and beginnings of egg coat (EC) are seen between oocyte (O) and follicle cells (FC). Whether this female had recently molted to maturity is not known.

most are usually bright orange and contain large oocytes filled with yolk (Figs. 6 and 7). In many cases these oocytes are still surrounded by follicle cells and may or may not show traces of an egg envelope (Figs. 6 and 7). Thus, these oocytes apparently overwinter until the next breeding season commences the following spring and are released as the first brood of the year. Mature females shipped to Florida from Woods Hole during the winter months have well developed ovaries and some days after acclimatizing to the warmer water temperature oviposit.

Seminal receptacles are absent in the immature females and very small in those newly molted to adulthood. In addition, newly molted adult females have not been observed mating in the soft condition.

Discussion

Arthropod metamorphosis, particularly phases of molting and sexual maturation, have been extensively studied in recent years. It is particularly well documented in the insects. Although crustaceans have been studied less extensively, many similarities to the insects have been noted. Ecdysis is controlled by neurosection from the X-organ (crustaceans) of the eyestalk or protocerebrum (insects). This neurosecretion activates the Y-organ (crustaceans) or corpora cardiaca (insects) to produce a hormone which initiates molting. Molting can be initiated in crustaceans by the removal of the eyestalk with its contained X-organ-sinus gland complex (See Passano, 1960; Highnam and Hill, 1969; Adiyodi and Adiyodi, 1970 for reviews). In *Libinia* the duration of time between eyestalk ablation and onset of molting varies and seems dependent on the relationship of time of ordinary ecdysis. Further factors may include temperature changes, nutrition, salinity and light-darkness periods as have been suggested by Aiken (1969) and Stephens (1952). Immature *Libinia* females molt following eyestalk ablation although mature crabs never have been seen to molt after reaching maturity. Thus *Libinia* like *Maja* (Drach, 1939) apparently undergoes a terminal molt to maturity. Perhaps this may be true of all members of the family Maijdae. In *Maja* (Carlisle and Knowles, 1957) failure to molt beyond the terminal stage has been attributed to the degeneration of the Y-organ. Whether the Y-organ degenerates in *Libinia* is not known.

Sex differentiation in crustaceans as in most animals is genetically determined although hormonally mediated. Sexual differentiation of the male is apparently controlled by the androgenic gland (Charniaux-Cotton, 1964; Charniaux-Cotton *et al.*, 1966). Females develop in the absence of the androgenic substance secreted by this gland. They can be masculinized by transplantation of the androgenic gland to their bodies. In most crustaceans, the reproductive cycles and body growth cycles are closely related and regulated by interlinked control mechanisms. These include the eyestalk hormones which control molting, oogenesis, vitellogenesis and secretion of sex hormones (for review see Adiyodi and Adiyodi, 1970) in many crustaceans having preadult and multiple adult female instars. Reproductive hormones influence the synthesis and accumulation of a sex-related lipoprotein utilized in vitellogenesis (Adiyodi, 1968a, b). Such a lipoprotein is found in mature female *Libinia* undergoing vitellogenesis but is absent in the blood of immature females and males (Bischoff and Telfer, University of Cincinnati and University of Pennsylvania, unpublished).

In *Libinia* sexual maturation of the females apparently occurs only following the terminal molt. Reproduction in this form can thus be studied independently of molting and the factors controlling sexual maturation recognized. Evidence presented here suggests some of the parameters of endocrine control of reproduction in *Libinia*. The eyestalk hormone apparently does not act to inhibit all stages of vitellogenesis. Evidence for this is the mature ovaries found in females out of the breeding season. In addition, the eyestalk hormone(s) seem to govern or regulate egg release. This is indicated by the continued oogenesis and oviposition beyond the normal season or resumption of oviposition following eyestalk ablation. The time delay between eyestalk removal and oviposition suggests that yet another factor which is controlled by the eyestalk hormone(s) may be involved. This could be a stimulating substance produced by the neurosecretory cells of the cerebral and/or thoracic ganglia (Ōtsu, 1963; Parameswaran, 1955) or could simply be the time needed for metabolic breakdown of the inhibiting hormone following eyestalk ablation. The fact that destalked immature females do not molt to maturity also implies hormonal control from a source other than the X-organ-sinus gland complex. The exact nature and origin of such factor(s) is yet to be determined. In some crustaceans the Y-organ has been suggested as a source of a gonad-maturation factor. However, if in *Libinia* the Y-organ degenerates as in *Maja* following the molt to maturity, then one must rule out such a role and look elsewhere.

Although the soft conditions following molting is generally considered favorable for mating in crustaceans it may be unimportant in *Libinia* since mating can occur in a hardened female (Hinsch, 1968). As reported here, newly molted mature females have not been observed to mate although such may occur under natural conditions. Males have not been seen carrying premolt females about as happens in other species, *e.g., Portunus* (Ryan, 1967), *Maja* (Schöne, 1968). The small size of the seminal receptacle in the newly molted adult *Libinia* suggests that the initial mating may occur much later in the hardened female. Hartnoll (1963) has reported that female *Maja* and *Pisa,* species belonging to the same superfamily as *Libinia,* are physically incapable of mating prior to the molt to puberty. This is the terminal molt. In general, ovarian maturation in members of this superfamily (*i.e., Pisa, Inachus, Hyas*) occurs after the molt to maturity (Hartnoll, 1963) as we have found in *Libinia.*

Summary

1. Carapace length is not sufficient for determining state of sexual maturation of female *Libinia.* Females in range of 4–6 cm carapace length may be mature or immature. Shape of the abdomen distinguishes between mature and immature females.

2. Eyesalk ablation of mature female *Libinia* results in extended periods of or initiation of the ovigerous state but does not appear to initiate molting. Breeding behavior and reproductve cycles seem unaltered by destalking.

3. Immature female *Libinia* which have had their eyestalks removed molt precociously but rarely to maturity.

4. *Libinia* apparently undergo a terminal molt to maturity. Ovarian development and vitellogenesis occur only in mature females.

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